Research Article

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Storage Duration Effect on the Nutritional Value of Freeze-Dried *Cleome gynandra* Leafy Vegetable

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ABSTRACT

The nutritive value of most stored leafy vegetables tends to be affected by storage duration. Cleome gynandra is a seasonal African traditional leafy vegetable with nutraceutical and pharmaceutical benefits. During its off-season, the vegetable could be dried and stored for two years to ensure its sustainability. However, the storage duration of dried vegetables could pose serious challenges in retaining their nutritional value. Forty days old tender leaves (1kg) of C. gynandra were harvested just before flowering and divided into seven samples. The first sample served as the control (fresh leaves), and the 6 samples were freeze-dried and then stored for 0, 2, 4, 6, 8, and 10 months, which served as treatments and were arranged in a completely randomised design in triplicate. Data collection included the essential elements, namely, nitrogen (N), sodium (Na), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), phosphorus (P) and zinc (Zn). These were subjected to analysis of variance at the probability level of 5%. Storage period had a significant ($P < 0.00$) effect on all measured nutritional components compared to the control. In all the tested mineral elements, their nutrient concentrations were increasing with storage duration, and the highest concentrations of 5.36 g/100 as well as 206, 1844, 199, 3113, 476, 623 and 5.17 mg/100g in N, Na, Ca, Fe, K, Mg, P and Zn, respectively, were achieved at 10 months of storage. In conclusion, the removal of moisture through freeze-drying retained the nutritive content of the leafy vegetable and helped improve its nutritive value by extending its storage life.

Keywords

Cleome gynandra, African leafy vegetables, Freeze-drying, Storage duration, Nutritional composition.

Introduction

Vegetables are considered a good source of nourishment since they contain vital phytochemicals, including fiber, vitamins, antioxidants, and minerals [1]. They are considered beneficial plant components that humans and certain animals can consume as edible plant matter. These edible plant parts include flowers, fruits, stems, leaves, roots, and seeds. It was reported that a healthy diet, which includes an adequate daily intake of green leafy vegetables, will help prevent major diseases such as cancer, heart disease, high blood pressure, high cholesterol, diabetes, and problems related to the prostate, among others [2-4]. Therefore, several nutritive minerals must be incorporated in daily diets in order to maintain optimum health [1].

Green leafy vegetables are categorized as exotic green leafy vegetables, namely spinach (*Spinacia oleracea* L.), cabbage (*Brassica oleracea*), broccoli (*Brassica oleracea var. Italica*), tomato (*Solanum lycopersicum*), onion (*Allium cepa*), as well as traditional green leafy vegetables, namely spider plant (*Cleome gynandra*), Amaranth (*Amaranthus spp.*), wilde jute (Corchorus *spp.*), etc [5]. Exotic vegetables are explained as those vegetables that are introduced to a region where they are not native, often cultivated under specific conditions to meet their unique growth requirements, whereas traditional leafy vegetables (TLVs) are those that either originate from the regions where they are cultivated or have been gradually integrated into local culinary traditions.

They are well adapted to the prevailing environmental conditions, making them resilient and sustainable for local agricultural systems [5]. In South Africa, most vegetable consumers prefer exotic green leafy vegetables, the reason being that they are easily accessible through commercial farming practices and are mostly researched based on their nutritional and medicinal value [6]. However, most individuals disregard the consumption of TLVs, viewing them as outdated, animal feed, "poor people's food," unhygienic foods, and even weeds to some [6]. Noticeably, TLVs are rich in essential nutrients, including calcium, iron, vitamins A and C, fiber, and protein. When compared to exotic vegetables such as cabbage (*Brassica oleracea*), these TLVs have superior levels of minerals and vitamins and are adapted to a wide range of environmental conditions [7], which gives them even more advantages over the exotic green leavy vegetables [8].

Amongst the TLVs, *Cleome gynandra* is reported to be one of the most widely consumed leafy vegetables, due to its nutrition and health promoting properties [9]. A study by Ventosa-Febles [10] reported that *C. gynandra* TLV, commonly known as the "spider plant" (English), "*gynandro*" (French), "pe hua tsai" (Chinese), "pambossie" (Somalia), "erotho" (Mauritius), and "nyevhe" (Zambia), is indigenous to South Africa. It has gained popularity for its high concentration of essential mineral elements, including iron, zinc, magnesium, calcium, sodium, phosphorus, and potassium [11,12]. The high nutritional content of macro- and micronutrients found in *C. gynandra* can be used as a preventative measure against malnutrition, obesity, and non-communicable diseases [9]. Research indicates that deficits of certain mineral elements affect up to two-thirds of the world's population in both industrialized and developing nations. These deficiencies might lead to negative health effects from inadequate mineral elements intake [1].

Cleome gynandra leafy vegetable is typically available during the summer season and mainly consumed fresh. However, during the off-season, it can still be made available to consumers as a dried leafy vegetable. Generally, fresh-plant-based foods may not be available all year round for consumption and the long-term storage of fresh foods could be challenging due to high water content, unavailability of cold-storage facilities (particularly in underdeveloped and developing countries), and the possibility of nutritional deterioration [13]. Consequently, drying of such foods may allow their long-term consumption and ease handling, transportation, and storage.

Generally, drying is a primitive technique used for food preservation, and it has been successfully applied in fruits and vegetables to minimize their biochemical, chemical and microbiological deterioration by reducing moisture to levels which allow safe storage over an extended duration [14]. This preservation technique can be achieved through various methods, including sun-drying, oven-drying, solar-drying and freeze-drying [15]. Like any other leafy vegetable, *C. gynandra,* if not adequately preserved immediately after harvest, spontaneous decay takes place. Freeze-drying, also known as lyophilization, is a well-known technique for the production of high-quality food powders and solids [16,17]. It is considered as one of the best methods of water removal, with final products of the highest quality. The solid state of water during freeze-drying protects the primary structure and the shape of the products with minimal volume reduction. In addition, the lower temperatures in the process allow maximal nutrient and bioactive compound retention [13].

Since most vegetables are seasonal and are abundantly available at certain periods of the year, a large amount of loss can occur during the growing season due to a lack of adequate storage facilities as well as the correct storage duration. Although *C. gynandra* can be made available as a dried leafy vegetable during the off-season, storage methods and duration pose challenges in maintaining its nutritional value. The optimal storage duration that could effectively preserve the nutritional content of freeze-dried *C. gynandra* has not yet been documented. Therefore, the objective of this study was to investigate the effect of storage duration under ambient temperatures on the nutritional value of freeze-dried *C. gynandra* leafy vegetables.

Materials and Methods

Description of the Study Area

Cleome gynandra plants were grown in a greenhouse environment at the Green Biotechnologies Research Centre of Excellence (GBRCE), University of Limpopo, Limpopo Province (23°53'10"S, 29°44'15"E), of South Africa (SA) during spring through to summer (September-December), 2023. The environment consists of warm and dry summers with maximum temperatures ranging from 28°C to 38°C, and summer rainfall of less than 500 mm. When the *C. gynandra* plants were at full vegetative stage, just before flower initiation [18], fresh tender leaves were then harvested into the brown bags and immediately transported to the Limpopo Agro-Food Technology Station (LATS) Food laboratory, University of Limpopo, Limpopo Province (23°53'10"S, 29°44'15"E) of SA for freeze-drying processing. Once the samples were freeze-dried, they were subjected to grinding in a Wiley-mill grinding machine (MF10 Basic, IKA WERKE, USA) to pass through a 1-mm-pore sieve [19], prior to nutritional analysis.

Treatments and Research Design

The non-freeze-dried freshly harvested *C. gynandra* leaves (control) and six storage durations (months) were used as treatments, namely, 0, 2, 4, 6, 8, and 10, which represented the duration of freeze-dried *C. gynandra* leaf powder in storage under room temperature. The treatments were laid out in a Complete Randomized Design (CRD) in triplicate (n=21).

Research Procedures

Cleome gynandra seeds were directly sown into the 25 cm diameter plastic pots filled with 100 ml of pasteurised (300°C) loamy soil mixed with compost at a ratio of 3:1 (v/v). Irrigation was done when the soil moisture level reached below 50% using tap water until termination at 40 days, when plants are at the full vegetative stage. Trellises of wooden rods and sisal strings were used to support tendril-vines when necessary. Forty days after establishing the experiment, fresh tender *C. gynandra* leaves at the full vegetative stage prior to flowering, were harvested in the morning and placed in brown paper bags and then transported to the LATS laboratory. Upon arrival at LATS, the samples were immediately stored in a refrigerator (model, KIC, SA) at −71°C for 2 hours to preserve their nutritional properties prior to freeze-drying.

Freeze-drying Procedures

Forty (40 g) portion of freshly harvested *C. gynandra* leaves were placed into a 50 ml centrifuge tube for freeze-drying (Figure 1a,b and c). Freeze-drying was performed using a Christ model: Alpha 1-2 LSC basic freeze-dryer (Separations, Osterode am Harz, Germany) with an ice condenser temperature set at −52.5°C and a vacuum pressure of 0.0565 mbar for 22 hours. After 22 hours of freeze-drying, samples were subjected to grinding in a Wiley-mill grinding machine (MF10 Basic, IKA WERKE, USA) to pass through a 1-mm-pore sieve (Figure 1d) [19].

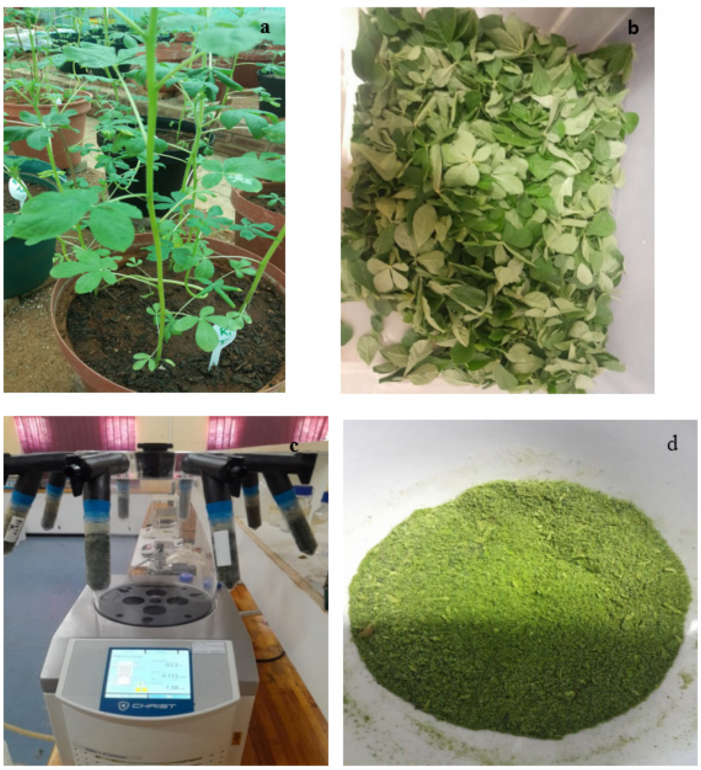

Figure 1: Schematic representation of *Cleome gynandra* plants grown under greenhouse conditions (a) and harvested fresh leaves at vegetative stage (b), freeze drying of the fresh (c) and freeze-dried *Cleome gynandra* (d).

The freeze-dried *C. gynandra* powder leaves were transferred into transparent airtight 40 ml baby food glass jars and placed in a dark cupboard at ambient temperature. The jars were covered with aluminium foil to protect the samples from light exposure and stored at ambient temperature for 0, 2, 4, 6, 8, and 10 months. Month zero (0) was considered the starting point of storage duration.

Data Collection

Determination of Nitrogen: Nitrogen analysis was conducted using a LECOTruSpec N nitrogen Determinator (Software Version 1.6x, Part Number 200-289). For each analysis, 0.2 g of the sample was accurately weighed using tin foil as a sample holder. The analysed results were recorded as nitrogen content (g/100 g).

Determination of essential mineral elements: A total of 7 samples, each replicated 3 times, were analysed. The fresh *C. gynandra* leaf (control) samples were the first to be analysed, followed by the freeze-dried leaf powder, each according to its storage duration. Each sample, weighing 400 mg, was digested using a PerkinElmer Titan MPS microwave digestion system with standard 75 ml digestion vessels. A digestion mixture consisting of 5.0 ml of Nitric acid (HNO_3, 70%) and 3.0 ml of Hydrogen peroxide (H_2O_2, 30%) was added to each sample. The mixture was carefully shaken to ensure thorough mixing. After a 10-minute reaction period, the digestion vessels were securely sealed and subjected to a controlled microwave heating program as shown in Table 1. The digestion process was expected to yield a clear solution with results expressed in mg/l [20]. The concentrations of selected essential mineral elements, including sodium (Na), calcium (Ca), iron (Fe), potassium (K), magnesium (Mg), phosphorus (P) and zinc (Zn), and, were determined, and the data were recorded accordingly.

Table 1: Nutritional analysis temperature program.

Step	Target temp (°C)	Pressure Max (bar)	Ramp-time (min)	Hold-time (min)	Power (%)
1	150	30	10	5	50
2	190	35	5	15	80
3	50	35	1	10	0
4	-	-	-	-	-
5	-	-	-	-	-

Data Analysis

Nutritional data of the analysed essential minerals were subjected to analysis of variance (ANOVA) using Statistics 10.0 software [21]. When the treatments were significant at the probability level of 5%, the associated mean sum of squares were partitioned to determine the percentage contribution of sources of variation to the total treatment variation (TTV) among the means. Mean separation was achieved using Fisher's Least Significant Difference (LSD). Unless otherwise stated, only treatment means significant at 5% level of probability were discussed.

Results

The storage duration had a highly significant ($p \leq 0.001$) effect on the nutritional composition (level) of the freeze-dried, stored *C. gynandra* TLV, contributing 98, 100, 99, 100, 100, 100, 99 and 98% to TTV in N, Na, Ca, Fe, K, Mg, P and Zn, respectively (Tables 2 and 3). Relative to the control, the lowest N concentration at storage duration of 0-month in freeze-dried *C. gynandra* TLV was 3.51 g/100 g and the highest N concentration was 5.36 g/100 g at 10-months storage duration, which was considered the highest duration (Table 3). However, the highest N concentration (5.36 g/100 g) was not significantly different ($p \geq 0.05$) from the N concentration (4.95 g/100 g) in freeze-dried *C. gynandra* TLV stored for 8-months (Table 4).

Similarly, the lowest Na concentration in freeze-dried *C. gynandra* TLV stored at 0-month was 45.50 mg/100 g and the highest Na concentration of 206.00 mg/100 g was achieved at 10-months storage duration, when compared to the control (Table 4). There were highly significant ($p \leq 0.00$) differences between the Na concentration of freeze-dried *C. gynandra* TLV stored from 0- to 10-months. In comparison to the control, the lowest Ca concentration in freeze-dried *C. gynandra* TLV was 1492.00 mg/100 g at 0-month storage duration and the highest Ca concentration at storage duration of 10-months was 1844.00 mg/100 g (Table 4). However, there was no significant ($p \geq 0.05$) differences between the highest Ca concentration (1844.00 g/100 g) of freeze-dried *C. gynandra* TLV stored for 10-months, with the Ca concentration of 1840.00 mg/100 g, 1811.00 mg/100 g and 1714.00 mg/100 g stored for 8, 6 and 4-months, respectively.

At 0-month storage duration, the lowest Fe concentration of 63.80 mg/100 g in freeze-dried *C. gynandra* TLV was achieved and the highest Fe concentration of 199.00 mg/100 g was obtained at 10-months storage duration, when compared to the control (Table 4). There were highly significant ($p \leq 0.00$) differences observed between Fe concentration of freeze-dried *C. gynandra* TLV stored for 0- to 10-months (Table 4). Relative to the control, the lowest K concentration of freeze-dried *C. gynandra* TLV was 1953.00 mg/100 g at storage duration of 0-month and the highest K concentration was 3113 mg/100 g at storage duration of 10-months (Table 4). However, there were no significant differences ($p \geq 0.05$) between the highest K concentration (3113.00 mg/100 g) at a 10-month storage duration and the K concentration of 3044.20 mg/100 g at 8-months storage duration.

On the other hand, the lowest Mg concentration of 287.00 mg/100 g was also achieved at 0-month storage duration in freeze-dried *C. gynandra* TLV, and the highest Mg concentration of 476.00 mg/100 g was obtained at 10-months storage duration, in comparison to the control (Table 4). There were highly significant differences ($p \leq 0.00$) between the Mg concentration of 476.00 mg/100 g stored at

Table 2: Mean sum of squares for nitrogen, sodium, calcium and iron of fresh and freeze-dried leaves of *Cleome gynandra* traditional leafy vegetable.

		Nitrogen (g/100 g)		Sodium (mg/100 g)		Calcium (mg/100 g)		Iron (mg/100 g)	
Source	**DF**	**MSS**	**%**	**MSS**	**%**	**MSS**	**%**	**MSS**	**%**
Treatment	6	5.12	98**	17797.90	100**	860389.00	99**	11614.20	100**
Error	14	0.10	2	2.90	0	11947.00	1	12.60	0
Total	20	5.22	100	17800.80	100	872336.00	100	11626.80	100

ns = non-significant at $p \geq 0.05$; * significant at $p \leq 0.05$, ** highly significant at $p \leq 0.00$.
DF = Degree of freedom
MSS = Mean sum of squares

Table 3: Mean sum of squares for potassium, magnesium, phosphorus and zinc of fresh and freeze-dried leaves of *Cleome gynandra* traditional leafy vegetable.

		Potassium (mg/100 g)		Magnesium (mg/100 g)		Phosphorus (mg/100 g)		Zinc (mg/100 g)	
Source	**DF**	**MSS**	**%**	**MSS**	**%**	**MSS**	**%**	**MSS**	**%**
Treatment	6	1833214.00	100**	45383.10	100**	70054.60	99**	4.31	98**
Error	14	4972.00	0	79.80	0	780.60	1	0.08	2
Total	20	1838186.00	100	45462.90	100	70835.20	100	4.39	100

ns = non-significant at $p \geq 0.05$; * significant at $p \leq 0.05$, ** highly significant at $p \leq 0.00$.
DF = Degree of freedom
MSS = Mean sum of squares

Table 4: Mean separation for nitrogen, sodium, calcium, iron, potassium, magnesium, phosphorous and zinc of fresh leaves (control) and freeze-dried (FD) leaves of *Cleome gynandra* traditional leafy vegetable.

Treatment	Nitrogen (g/100 g)	Sodium (mg/100 g)	Calcium (mg/100 g)	Iron (mg/100 g)	Potassium (mg/100 g)	Magnesium (mg/100 g)	Phosphorus (mg/100 g)	Zinc (mg/100 g)
	Variable^{Y}	**Variable**^{Y}	**Variable**^{Y}	**Variable**^{Y}	**Variable**^{Y}	**Variable**^{Y}	**Variable**^{Y}	Variable^{Y}
Control	1.50^{e}	38.00^{g}	342.13^{d}	48.70^{g}	955.00^{e}	122.00^{f}	211.00^{d}	1.90^{b}
FD-0 month	3.51^{d}	45.50^{f}	1492.00^{c}	63.80^{f}	1953.00^{d}	287.00^{e}	445.00^{c}	4.69^{a}
FD-2 months	4.30^{c}	50.10^{e}	1574.00^{bc}	88.20^{e}	2731.00^{c}	353.00^{d}	539.21^{b}	5.00^{a}
FD-4 months	4.70^{bc}	169.00^{d}	1714.00^{ab}	153.00^{d}	2832.00^{bc}	400.00^{c}	612.00^{a}	5.10^{a}
FD-6 months	4.76^{bc}	177.00^{c}	1811.00^{a}	170.00^{c}	2880.30^{b}	439.33^{b}	621.33^{a}	5.12^{a}
FD-8 months	4.95^{ab}	195.00^{b}	1840.00^{a}	190.00^{b}	3044.20^{a}	446.00^{b}	621.33^{a}	5.16^{a}
FD-10 months	5.36^{a}	206.00^{a}	1844.00^{a}	199.00^{a}	3113.00^{a}	476.00^{a}	623.00^{a}	5.17^{a}

^{Y}Column means with the same letter were not significantly different $p \geq 0.05$ according to Fischer's Least Significance Difference test.

10 months of storage duration and the Mg concentration of 446.00 mg/100 g and 439.33 mg/100 g stored at 8 and 6-months of storage duration, respectively (Table 4).

In comparison to the control, the lowest P concentration was 445.00 mg/100 g at 0-month storage duration in freeze-dried *C. gynandra* TLV, and the highest P concentration of 623 mg/100 g was again noticed at 10-months of storage duration (Table 4). However, there was no significant differences ($p \geq 0.05$) between the highest P concentration (623.00 mg/100 g) at 10-months storage duration and the P concentrations of 621.33 mg/100 g, 621.33 mg/100 g and 612.00 mg/100 g at 8, 6 and 4-months, respectively.

With Zn nutrient element, relative to the control, the lowest Zn concentration was 4.69 mg/100 g, also at 0-month of storage duration in freeze-dried *C. gynandra* TLV, and the highest P concentration was 5.17 mg/100 g achieved at 10-months of storage duration (Table 4). There were no significant differences ($p \geq 0.05$) observed between Zn concentrations at 0- and 10-month storage durations.

Discussion

Overall, in this study, storage duration demonstrated significant ($p \leq 0.00$) effects on the tested nutritional elements in freeze-dried *C. gynandra* TLV, namely, N, Na, Ca, Fe, K, Mg, P and Zn. Basically, *C. gynandra* leafy vegetable is known to be abundant in essential minerals such as P, K, Ca, Mg, S, Fe, Mn, Cu and Zn, which are not only important for the proper growth and development in humans and animals, but as well as for the prevention and treatment of diseases [22]. Notably, the tested nutritional elements of freeze-dried *C. gynandra* in storage, increased at an increasing rate with the experimented storage duration (0 – 10 months), when compared to the nutrients observed at the control (fresh leaves). It was also observed that the freeze-dried *C. gynandra* TLV stored for 10-months (which was considered the longest in this study) were found to have the highest nutritional composition of all the nutrient elements tested, indicating that freeze drying, paired with the storage of the leafy vegetable during off-season is advantageous to the consumer nutrition. However, the availability and quantity of certain nutrients statistically behaved differently at different storage duration.

The N concentration at storage duration of 8- and 10-months didn't show any significant differences ($p \geq 0.05$), similarly to the K, which also didn't show any significant differences ($p \geq 0.05$) at storage duration of 8- and 10-months. However, both their concentrations increased with storage duration, which is important for the end-users of the leafy vegetable. Nitrogen is an essential nutritional element, and it is considered highly important to all living material, whereby plants, animals and humans cannot function properly without it. Suzuki et al. [23] reported that in human beings, the daily N intake requirement (104.2 mg N/kg) was not gender or age related (below or above 60 years of age). Generally, N forms a major part of amino acids, which are the building blocks of proteins as well as nucleic acids such as DNA, which is important in transferring genetic information to subsequent generations of organisms [24,25]. In the current study, the N concentration in stored freeze-dried *C. gynandra* TLV was above the recommended daily intake (104.2 mg N/kg) as stated by Suzuki et al. [23], indicating that the N in freeze-dried and stored *C. gynandra* TLV can be a good source of nutrition in humans during the off season.

On the other hand, Shonte et al. [26] alluded that, the type of drying method used could have an impact on the specific nutrient's degradation and retention. Agomuo et al. [27] observed an increase in K concentration during drying using different drying methods, such as shade, oven and sun drying, incidentally the same was observed in this study when the TLV was freeze-dried prior to storage. The retention and increase of K nutrient element also play a significant role in human body. Several research studies have demonstrated that a diet rich in K reduces blood pressure in people suffering from high blood pressure, lowers mortality from cardiovascular disease, potentially prevent or slow the progression of kidney disease, as increased K intake reduces urinary Ca excretion and plays a crucial role in managing hypercalciuria and kidney stones, likely lowering the risk of osteoporosis [28-32]. According to Harvard [33] the daily recommended intake of K is 2,300 mg for teenagers between the age of 14-18 years and 2,600 mg for women of 19+ years of age. For men, the daily recommended K intake is 3,000 mg between the age of 14-18 years and 3,400 mg for 19+ years of age. Compared to the findings in this study, the K of stored freeze-dried *C. gynandra* TLV, contained enough K concentration that exceed the daily recommended intake, except for older men aged 19+ years, whereby an additional 287 mg would be required to meet the daily recommendation intake. Therefore, freeze-dried *C. gynandra* TLV stored up to 10 months can serve as a good source of K nutrient, for consumers.

The concentrations of Na, Fe and Mg also showed some similar patterns in all the tested storage duration, which showed significant increase in the nutrient concentrations with increased storage duration of freeze-dried *C. gynandra* TLV. Although studies on the effect of storage on the nutrient elements of freeze-dried TLVs are scanty, recently Isaac [34] reported that drying methods (shade, freeze and oven drying) can increase the concentration of nutrients of the dried vegetables. In his study, it was speculated that the increase in Na, might be due to the moisture loss, which then resulted in higher accumulation or concentration of the tested nutrient element. Garba and Oviosa [35] also indicated that the removal of moisture through heat, generally enhance the digestibility of foods, increases the concentration of nutrients and may also increase the availability of certain nutrients. Sodium is regarded as one of the essential elements in the human body. When paired with K, these two nutrient elements were reported to be vital for proper body functioning [36]. Recently Mohamed et al. [37] reported that Na plays a vital role in regulating blood pressure and extracellular fluid volume, as well as in the resting potential of the heart. It is also crucial for the transmission of the nerve signals and in muscle contraction, ultimately affecting neuromuscular function. Awuchi et al. [38] recommended that the daily intake allowance of Na in adults (irrespective of gender), with age ranging between 31-50 years should be 1500 mg, with a tolerable upper intake level of 2300 mg. On the other hand, the National Health and Medical Research Council of Australia (NHMRC) [39] recommend 920 mg daily intake of Na for adults. Both the recommended ranges fall above the Na concentration values that was contained in the freeze-dried *C. gynandra* at all the tested storage durations in this study, therefore abundant consumption of *C. gynandra* is recommended to meet the daily required Na intake amount.

Iron is considered one of the vital elements in many living organisms due to its involvement in various metabolic processes, such as oxygen transport, DNA synthesis, electron transport and as well as to produce the red blood cells [40,41]. The increase in Fe concentration in freeze-dried and stored *C. gynandra* TLV, was supported by a study of [42]. Oulaï et al. [42] observed an increase in Fe concentration of shadow-dried of five leafy vegetables species (*Amaranthus hybridus, Andasonia digitata, Ceiba patendra, Hibiscus sabdariffa and Vigna unguiculata*) widely consumed in Northern Côte d'Ivoire, during the storage duration of 15 days. Garba and Oviosa [35], also observed an increase in Fe concentration while using different methods of drying in *Vernonia amygdalina* leafy vegetable. As a result, the concentration of Fe in freeze-dried and stored *C. gynandra* TLV in this study, was found to be enough for the recommended daily requirements as indicated by [33]. The study recommended a daily intake of 18 mg (19 – 50 years) and 8 mg (50+ years) in women, respectively, whereas in men, a daily intake of 8 mg at age 19 to 50+ years is advised. In this study, the stored freeze-dried TLV can be able to meet the daily Fe requirements.

The highest Mg concentration of freeze-dried *C. gynandra* TLV, was also observed to increase with storage duration and the highest was observed on the 10th month of storage. These results correspond with findings by Oulaï et al. [42] who observed an increase of Mg concentration in storage of the shadow-dried-five leafy vegetables species widely consumed in Northern Côte d'Ivoire. Similar, to the other elements, Mg performs various essential roles in the human body. It serves as a cofactor for over 300 enzymes, regulating various essential functions like muscle contraction, neuromuscular conduction, glycemic control, myocardial contraction and blood pressure management. Furthermore, it also has an essential function in energy generation, active transmembrane transport of other ions, synthesis of nuclear materials and bone development [43,44]. Generally, the recommended daily intake of Mg is 310 mg and 320 mg for women at the age of 19-30 years and 31-70+ years, respectively, whereas for men, the recommended daily intake is 400 mg and 420 mg for men aged 19-30 years and 31-70+ years, respectively [33]. The Mg concentration of freeze-dried *C. gynandra* TLV observed on the 6th, 8th and 10th month of storage in this study surpasses the daily recommended intake, therefore the Mg concentration in *C. gynandra* could be a good source of daily Mg intake requirements.

The Ca and P concentration in freeze-dried *C. gynandra* TLV also had similarities in response to the storage duration effect. Although their concentrations were increasing with storage duration, the two nutrient elements demonstrated no significant ($P \leq 0.00$) differences at storage durations of 4- through to 10-months. Oulaï et al. [42] also reported an increase in Ca concentration in shadow-dried leafy vegetables species in storage. Several studies on African traditional vegetables had reported an increase in Ca concentration when different drying methods were used, such as air, sun, oven, solar and freeze-drying methods [34,35,45]. The nutrient element, Ca is considered the most abundant mineral found in the human body, about 1kg, of which more than 99% is stored in the bones as calcium phosphate [46]. A study by Harvard [33] indicated that the daily Ca intakes vary according to the age groups and gender, which recommends 1000 mg for women aged 19-50 years and 1200 mg for women aged 51+ years. For men, a daily intake 1000 mg is recommended for those aged 19-70 years and 1200 mg for the elderly (71+years). Calcium is beneficial in muscle contraction, oocyte activation, blood dotting, building strong bones and teeth, nerve impulse, transmission, in the regulation of heartbeat and in fluid balance within cells. *Cleome gynandra* TLV is rich in minerals concentration and is recommended for high consumption, due to its ability to contribute to malnutrition and micronutrients deficiency problems [47]. The Ca concentration at in this study can supply the daily required intake, therefore stored freeze-dried *C. gynandra* TLV is a good source of Ca recommended for daily intake for human being.

With regard to P concentration of freeze-dried and stored *C. gynandra* TLV, no significant ($P \geq 0.05$) difference were observed when the vegetable was stored for more than 4 months, however, the nutrient concentration increased with storage duration. Agomuo et al., [27] also observed an increase in P in dried leafy vegetables under the application of different drying methods, namely, shade, oven and sun drying. Storage of vegetables is essential especially to enhance their availability during the gap season and in the promotion of food security. A meal rich in P performs important functions in both skeletal and non-skeletal tissues and is essential for the production of energy in human being. It is regarded one of the vital nutrients, functioning as a signalling molecule and triggering complex physiological responses [48]. The daily intake of 700 mg is recommended for both women and men, with upper limits of 4000 mg for adults aged 19-70 years and 3000 mg for 71+ years [33]. Looking at the P concentration of stored freeze-dried *C. gynandra* in this study, the obtained values is lower than the recommended daily intake, therefore, to achieve daily recommendation, an additional of 77 mg intake during consumption is recommended for freeze-dried *C. gynandra* TLV at storage duration of 10 month.

Regarding the Zn concentration in freeze-dried *C. gynandra* TLV at all the tested storage duration (0- to 10-months), no significance ($P \geq 0.05$) differences was observed, except when compared to the fresh leaves at the control. Freeze-dried *C. gynandra* was able to retain the Zn concentration (around 5.00 mg/100 g) irrespective of the storage duration. Zinc ranks second to Iron as the most prevalent micronutrient in the human body and it is deemed extremely vital for a properly functioning immune system and overall human health. It plays a crucial role in various biological processes within the human body, including the regulation of cell differentiation, proliferation, and apoptosis, which impact organism growth [49, 50]. The daily recommended Zn intake is 8 mg and 11 mg for women and men, respectively, whereas the upper limit is 40 mg [33]. The Zn concentration of stored freeze-dried *C. gynandra* in this study does not amount to the daily recommended intake as recommended by Harvard [33] however, the TLV can still supply around 5.00 mg/100 g.

Conclusion

Findings in this study demonstrated that the various storage durations had an effect on the tested nutritional elements of freeze-dried *C. gynandra* leafy vegetable. Therefore, the null hypothesis of this objective is rejected as the nutritional composition of freeze-dried *C. gynandra* TLV was increasing with increasing storage duration. All the tested storage duration were suitable to maintain the nutritive value of *C. gynandra*, with the 10-month storage duration being the best in retaining the increased nutritive concentration of all the important nutritive elements. It is recommended that the TLV should be stored in order to supplement reduction during the gap season.

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